CRUDE OIL BIOREMEDIATION IN ARCTIC SEASHORE SEDIMENTS

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Abstract

Oil is an important energy source but also an environment pollutant. Crude oil spills along arctic shorelines might occur due to the expected increase in offshore oil production. To reduce adverse effects on the environment in the case of a spill, it is important to develop approaches to remove spilled oil. Bioremediation with addition of nutrients has shown promising results in enhancing oil degradation rates. This research focuses on determining the effect of different environmental conditions on the rate of crude oil biodegradation in laboratory experiments, as a proxy for oil spills at arctic seashores. Laboratory microcosms were set up containing beach sediments collected from Barrow, spiked with North Slope Crude. These microcosms were incubated at varying temperatures (3°C vs. 20°C), salinities (30 vs. 35 g/L) and crude oil concentrations (1 vs. 5 mL/kg), all with a standard concentration of nutrients. Measurements of respiration rates (breakdown of hydrocarbons to CO_2), hydrocarbons remaining in the sediment (GC/FID), and hydrocarbons volatilized and sorbed to activated carbon (GC/MS) were performed. In all microcosms, higher respiration rates by naturally occurring microorganisms were observed at 20°C compared to 3°C. Surprisingly, volatile organic compounds (VOC) release was similar at both temperatures, for different crude oil concentration and salinities. High total petroleum hydrocarbon (TPH) levels remained at 3°C for microcosms with high initial crude oil concentration. Regardless of temperature, increased salinity had a positive impact on the rate of crude oil removal, i.e. high CO_2 release, high VOC production and low amount of TPH in sediments. At higher crude oil dosages, a larger amount of volatiles was released, however CO_2 production did not significantly increase with the contaminant concentration. The results of this study will assist decision-makers in choosing effective spill response strategies for future crude oil spills in Arctic shorelines.

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Chapter 1 Introduction

1.1 Background

1.1.1 Oil exploration in the Arctic

Petroleum products are the main energy source for transportation worldwide. Various petroleum products are derived from crude oil. Crude oil demand has increased with the population explosion. Countries are searching for oil reserves around the globe to meet this increasing demand.

According to a USGS study, the Arctic region could hold approximately 13% of the world's undiscovered crude oil (EY 2011). Russia holds the largest natural gas resources, whereas the U.S holds largest arctic oil reserves in Alaska (EY 2011). With Alaska being the hub of oil reserves, oil companies are interested in investing in oil exploration in Alaska's Arctic. Most of the North Slope region of Alaska is still unexplored and has immense potential for future exploration (Bélanger, 2007). In December of 1967, North America's largest oil field was discovered (ADA 2011). It is estimated that the Alaskan Arctic region contains about 30 billion barrels (EY 2011). BP estimates 25 billion barrels of oil, of which 11.3 billion barrels had been produced by February 2011 (ADA 2011). The Alaskan Arctic region can be divided into five areas: the Arctic National Wildlife Refuge (ANWR), the Central Arctic, the National Petroleum Reserve-Alaska (NPRA), the Beaufort Outer Continental Shelf (OCS) and the Chukchi Sea.

Companies are planning to approach Arctic waters for more oil exploration. For example Royal Dutch Shell was approved by EPA to drill oil and gas wells in the Beaufort and Chukchi seas in mid-February 2012 and has been moving the drilling rig Polar Pioneer to Alaska in the summer of 2015. The decline in on-shore oil production could be compensated by offshore oil production mainly in the Beaufort Sea and Chukchi Sea. It is anticipated that offshore drilling in the Beaufort Sea could produce its first oil in 2019, with a cumulative volume of 5 billion barrels of oil.

Similarly, the first oil may be produced from the Chukchi Sea in 2022, with a cumulative volume of 4.8 billion barrels of oil (EY 2011, ISER 2009).

These oil explorations could bring large revenues to the respective countries and profits to the concerned oil companies. Offshore exploration would bring a total payroll of $72 billion over a 50 year period and an annual average increase of 6% in the job opportunities for the local residents. In coming years, there will be a rise in the offshore drilling operations in the Arctic (EY 2011, ISER 2009).

1.1.2 Risk of oil spills in the Arctic

Increased exploratory drilling in the Chukchi and Beaufort Seas (ISER 2009) can increase the risk of oil spills in Alaska's arctic marine waters. Though economic benefits of oil production will support the local communities, oil spill accidents have the potential to adversely affect the ecosystem and the livelihood of the local population, especially when it is based on subsistence fishing. From 1996-1999 an average of 407 spills of oil products has occurred in Alaska annually and 19% of the incidents took place in permafrost regions (Zong et al. 2009).

The Arctic Ocean is one of the world's least explored marine ecosystems, being a habitat for many species such as polar bears, ice seals and walruses. The arctic tundra is complex and consists of peat mats, brackish lagoons, tundra and some other vegetation (CRRC 2010). It would be a disaster if an oil spill occurred in Arctic waters and reached its shorelines. A small section of the total Arctic shoreline, the coastline lengths of the American Chukchi and Beaufort seas are 4662 km and 3376 km respectively (Lantuit et al. 2011). Some of the Beaufort Sea area consists of brackish lagoons. The Beaufort Sea coast vegetation is dominated by wetland plants and wet sedge and moss communities (CRRC 2010). The Beaufort and Chukchi seas are predominantly frozen for about eight to nine months in a year. During the summer, the water temperature varies from 5 to

10°C and salinity varies from 10 to 25% (CRRC 2010). The average summer temperature in the Barrow region is approximately 3 degree Celsius and during winter the average temperature drops to -45 degree Celsius (CRRC 2010). This region also experiences strong winds which contribute to soil erosion, change nutrient availability, and endanger community and industry infrastructure (Lantuit et al. 2011, CRRC 2010).

Oil spills endanger rivers, coastline and terrestrial habitats, especially at oil drilling, refining and transport sites. These spills also affect the public, government agencies and people involved in clean-up (Miraglia, 2002). In the 1989 Exxon Valdez oil spill accident, 11 million gallons of oil spilled in Prince William Sound (Cheremisinoff and Rosenfeld, 2009). This accident flooded sea bird and harbor seal habitat in oil (Rice et al. 2007). The oil has persisted in some locations for decades (Rice et al. 2007). Exxon Valdez, Deepwater horizon and other oil spills are examples that demonstrate the threat of oil exploration and development. The commercial benefits of increased oil revenues must be weighed against the potential environmental costs of oil spills.

Declining sea ice facilitates oil exploration in the Arctic, increasing the risk of an oil spill. On-shore and near shore sediments are usually contaminated when an oil spill occurs near land (Heiser, 1999). Extreme conditions, poor infrastructure and vulnerable ecosystems in the Arctic will make oil spill response difficult (Sydnes and Sydnes, 2013). Unfortunately, the Arctic coastal dynamics have not been studied much; therefore a thorough understanding of Arctic coasts is necessary. Due to its remoteness and harsh weather, Alaska's Arctic coast has limited infrastructure such as ports, roads and airports (CRRC 2010). With on-going oil exploration in this region, if an oil spill occurs, there is minimal oil spill response equipment. As a result, the Arctic is not equipped and ready for any oil spill. Therefore, it becomes important to investigate appropriate oil spill response strategies in such conditions, which is the focus of this thesis.

1.1.3 Oil spill management in the Arctic

Overall management of oil spills is increasing in complexity and magnitude worldwide. There are various spill response techniques to clean-up an oil spill. The most effective spill response depends on a number of factors such as type of oil (viscosity, composition), geology, amount of turbulent energy, temperature, sea and air currents, sensitivity of biological communities and water salinity (EPA 2014, Owens and Lee, 2003). There are many factors to consider before recommending an oil spill response for a particular place. Rapid removal of spilled oil is important in order to reduce the harmful effects of oil spills on sensitive habitats (EPA 2014).

Clean-up of oil spills in Arctic waters poses great challenges because of harsh conditions. Inadequate infrastructure and harsh weather can delay the arrival of additional vessels, equipment and other supplies (PEW 2013). The presence of ice confounds clean-up efforts by interfering with mechanical oil removal (PEW 2013). It is critical to identify technologies and/or recovery techniques specifically tailored to oil spill clean-up in Arctic waters.

1.1.4 Oil spill cleanup methods

There are mechanical, chemical and biological methods for oil spill response. Mechanical methods include the use of booms, skimmers and other sorbent barriers. Mostly inflatable booms are used to concentrate the oil on the water surface for its easy recovery. Skimmers suck up the oil from the water surface and sorbent materials absorb the oil (EPA 2014). Chemical methods include the use of dispersants to break down the oil into tiny droplets allowing more surface area for microbes to act on them (EPA 2014). Herders are chemicals that are used to thicken the oil spills such that they can undergo in-situ burning. Biological methods rely on microbial degradation for removing the spilled oil. A combination of mechanical removal and bioremediation, enhanced by nutrient

addition, has shown promising potential in clean-up efforts in the past, for example after the Exxon Valdez oil spill (Wrabel and Peckol, 2000).

Biodegradation as an oil attenuation process in cold environments is well documented in many research papers (Brakstad and Bonaunet, 2006). Biodegradation can be the cheapest and the most environmentally beneficial approach for removing hydrocarbons (Prince et al. 2003). Complete removal from rocks and sandy areas is hard to achieve by physical methods in combination with biodegradation (Fernández-Álvarez et al. 2006). *In situ* bioremediation can be the method of choice in remote locations and where mechanical removal is not feasible.

Biodegradation is the break-down of complex compounds into simple molecules by microbes. The terms bioremediation and biodegradation are sometimes used interchangeably, however they are not synonyms; the term biodegradation is broader than bioremediation. Bioremediation is a specific case of biodegradation, where it is used as an engineered method to degrade undesired complex compounds. Bioremediation includes bio-stimulation and bio-augmentation. Bio-stimulation is the addition of nutrients or electron acceptors to accelerate the growth and metabolic rate of micro-organisms. Bio-augmentation is the addition of oil-degrading microbes at the contaminated region to increase the rate of biodegradation.

Bioremediation can be a cost effective and safe technique for the clean-up of crude oil contaminated sediments, even in Arctic and subarctic environments (León et al. 1998, Aislabie et al. 2006). However some previous studies have shown that bioremediation was not effective for heavy crude oil and in low temperature environments as in Alaska (Brakstad and Bonaunet, 2006, Gibb et al. 2001). Further research is necessary to evaluate under which conditions bioremediation of crude oil can be effective in cold climates. The oil biodegradation rate can be increased by adding nutrients, however temperature as a limiting factor in degradation cannot be controlled as

easily, especially for larger spills (Heiser, 1999, Gibb et al. 2001). Oil spill bioremediation can be successful with nutrient supply even at low temperatures (Heiser, 1999, Gibb et al. 2001). Several researchers have concluded that nutrient supply and adjustment of pH, oxygen and soil moisture levels can increase the oil biodegradation rates in Alaskan soils (Rice et al. 2007, Cheremisinoff and Rosenfeld, 2009, Heiser, 1999, Horel and Schiewer, 2009). Oil spilled on a shoreline penetrates into the sediments. The depth of penetration of oil into the sediments depends on the viscosity of the oil and the sediment characteristics (Zong et al. 2009).

Fortunately, Arctic shorelines have not yet been exposed to a major oil spill, thus little is known about biodegradation of crude oil in that environment. Limited research has been conducted on crude oil degradation at low temperatures in soil and on the effect of salinity (Minai-Tehrani et al. 2009). Therefore, it is essential to study the rate of crude oil biodegradation in arctic seashore sediments. However, sometimes results of small scale laboratory studies cannot directly be scaled up due to heterogeneity and concentration gradients in larger settings (Horel et al. 2015).

1.2 Objectives

This thesis focuses on evaluating the potential for crude oil biodegradation in an oil spill response along Arctic shorelines. The objective of this study was to investigate the combined effects of varying temperatures, crude oil concentrations and salinities on crude oil biodegradation and fate in laboratory microcosms, simulating environmental conditions along Alaska's arctic shore. Biodegradation was studied for Barrow beach sediments as a proxy for an actual oil spill bioremediation on the Arctic shoreline. Typical sand/gravel beach sediments obtained from Barrow were spiked with a known volume of crude oil as contaminant, amended with nutrients, and incubated to determine to what extent crude oil was degraded, volatilized or remained in the

sediments. The research will assist decision-makers in choosing effective spill response strategies for future crude oil spills in Arctic shorelines.

1.3 Hypotheses

1. Crude oil is degraded by indigenous microbes present in the Barrow sediments (i.e. biostimulation or inoculation with microbes is not required).
2. Mineralization of crude oil increases with increasing contaminant concentration in absolute terms, but relative mineralization percentages will be lower for higher crude oil concentrations.
3. Increasing crude oil concentrations leads to higher volatilization in absolute terms.
4. For higher crude oil concentrations, higher quantities of crude oil will remain in the sediment.
5. Biodegradation is faster at higher temperatures, but even at low temperatures measurable degradation (CO_2 production) will take place over the course of several months.
6. Volatilization is greater at higher temperatures.
7. Overall hydrocarbon removal from soil will be greater at higher temperatures.
8. Higher salinity will have a positive impact on hydrocarbon degradation rates (CO_2 production).

Chapter 2 Literature Review

2.1 Impact of different factors on biodegradation

The rate of hydrocarbon biodegradation depends on many different factors such as nutrient availability, microbial growth, oxygen, water content, sediment type, temperature, hydrocarbon type, pH-value and bioavailability of contaminants (Margesin 2000). For any oil spill all environmental factors influence the degradation rate cumulatively. Bioremediation accelerates the natural attenuation via optimizing the limiting environmental conditions present at a spill site (Margesin 2000). Some of the factors particularly relevant for the present research will be discussed in the following subsections.

2.1.1 Microbial potential

According to the Bass Becking principle "everything is everywhere, but the environment selects" (De Wit and Bouvier, 2006). This statement means that microbes will grow and flourish where suitable environmental conditions are present, which can be a combination of nutrients, temperature and biogenetic potential. Microbial growth generally follows a non-linear growth curve with four different stages.

1. Lag phase

   In this phase, microbes start to adapt to the new environment (in this case one where crude oil is present as a substrate) and microbial activity is low. The duration of this phase may vary from hours to weeks depending on many other factors such as temperature. Bioremediation would be most efficient if this phase is brief.

2. Log phase/Exponential phase

   The exponential phase follows the lag phase. During this phase, the number of microbes increases exponentially with rapid growth and substrate degradation. The longer the exponential phase can be maintained, the more effective the bioremediation.

3. Stationary phase

   The stationary phase commences when either the substrate concentration has declined or the death rate starts to balance the growth rate.

4. Death phase

   At this stage the death rate exceeds the growth rate, due to a combination of substrate limitations and/or toxin accumulation. In this phase, the microbial population drastically declines.

Figure 2.1 describes microbial growth over time under suitable conditions. The growth of microbes is sectioned into three phases in this figure, with no death phase being shown. The first derivative curve shows the growth rate, based on a first order reaction.

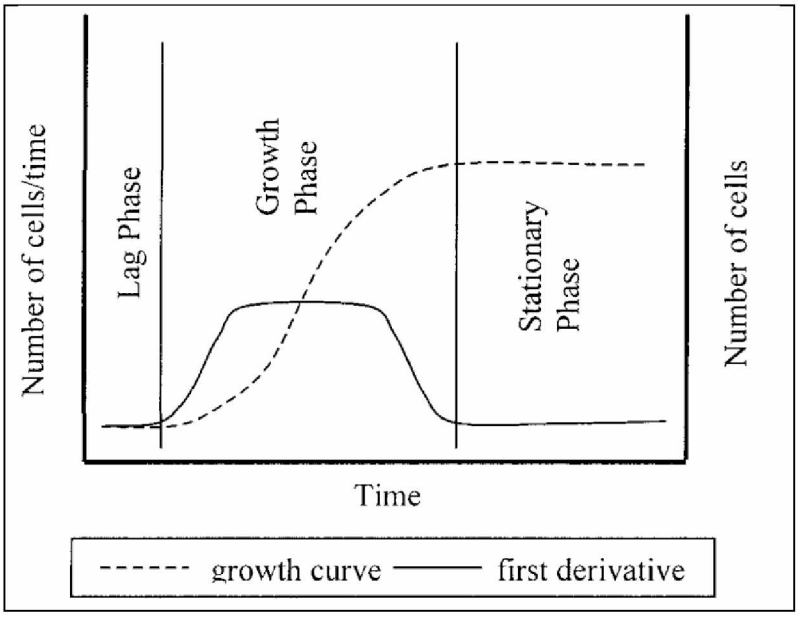

Figure 2.1 Microbial growth phases (Gibb et al. 2001).

2.1.2 Role of nutrients in hydrocarbon degradation at low temperatures

Microbial growth would not be possible without suitable nutrients present in the system. Microbes require a source of carbon (main substrate, e.g. hydrocarbon), sources of nitrogen and phosphorus (nutrients) as well as electron donor/acceptor for microbial growth (Zong et al. 2009). In aerobic respiration, heterotrophic microorganisms use oxygen as a terminal electron acceptor (Rike et al. 2003). Nitrogen and phosphorus are often limiting nutrients in Arctic soils and therefore their supplementation enhances the degradation of hydrocarbons. Addition of a commercial fertilizer such as 20:20:20 increased the mineralization of the majority of crude oil alkanes in Arctic soils (Aislabie et al. 2006). Gravel-sandy soil of Barrow, Alaska was amended with 50-100 mg N/kg; this increased the hydrocarbon degradation whereas 200 mg N/kg inhibited the degradation activity (Aislabie et al. 2006). This inhibition of soil microbial activity could be due to nitrite toxicity (Zong et al. 2009). Redfield stoichiometry states that the desired ratio of C, N, P and K is 100:15:1:1 (Zong et al. 2009). Therefore, it is important to supply nutrients in the form of N and P at any temperature as they often become limiting especially when the contaminant functions as a carbon source (Zong et al. 2009).

Nutrient addition to oil polluted sites facilitates faster microbial growth and thus hydrocarbon degradation. A field study was conducted with an old oil-contaminated soil in a skiing resort area, where fertilization lead to 42% reduction in hydrocarbon contamination, whereas natural attenuation lead to a reduction of only 14% of oil initially present in the soil from motor vehicles oil leakage (Margesin, 2000). Similar studies at low temperatures have demonstrated a positive relationship between degradation of oil and nutrient supply (Heiser, 1999). In a cold alpine soil contaminated with diesel, fertilizer addition led to 43% decontamination in 30 days.

2.1.3 Role of temperature

Temperature plays an important role in the process of biodegradation and bioremediation. Biodegradation typically follows the Arrhenius relationship, where metabolic activity decreases with a decrease in temperature (Heiser, 1999, Zong et al. 2009). The Arrhenius relationship can also be applied to microbial community systems. The growth of microbes is dependent on the temperature and with an increase in temperature microbial growth increases. A study showed that heavy fuel biodegradation in the North Sea was four times faster at 18°C than at 4°C (Zong et al. 2009). The microbial activity at low temperatures slows down but it does not cease (Zong et al. 2009, Aislabie et al. 2006). Laboratory experiments have shown that, though the microbial activity does not cease in a cold environment, the rate of mineralization is still higher in warmer environments (Aislabie et al. 2006, Horel and Schiewer 2011, Schiewer and Niemeyer 2006). Despite the cold, hydrocarbon-degrading microorganisms occur and are able to survive solely on hydrocarbon products (Rike et al. 2003, Heiser, 1999, Zong et al. 2009).

Crude oil is a complex mixture of different hydrocarbons and its properties depend on the surrounding temperature (Heiser, 1999, Zong et al. 2009). Temperature is a critical parameter for bioremediation as it affects the rate of hydrocarbon degradation, microbial growth and mass transfer of substrate in cold soils (Zong et al. 2009). At low temperatures, the alkanes in crude oil become less volatile and more water soluble thus prolonging the degradation process. On the other hand, some of the hydrocarbons become less water soluble which allows the microbial community to degrade those hydrocarbons (Heiser, 1999).

2.1.4 Role of sediment characteristics

Sediment characteristics are also an important factor in determining the fate of crude oil in sediments. Every beach is different in terms of climate, grain size distribution and biological and

chemical characteristics. The components of the beach sediments govern the possible effects of oil on the shoreline (EPA 2014). The environmental sensitivity index [ESI] classifies the susceptibility to oil spills on the basis of three factors: shoreline classification, biological resources and human use resources (NOAA 2002). ESI maps are a vital part for oil spill response and planning since 1979. According to the beach sediment profile and biological resources present at the Chukchi Sea and Beaufort Sea, much of these areas falls under the ESI 5B category, which applies to mixed sand with at least 20% gravel with an intermediate slope of 8-15 degrees. The fauna and epifauna populations are very low (NOAA 2002). The eastern Beaufort Sea coast sediments contain a large amount of organic carbon due to river inputs and coastal erosion of peat. However, the fate of this organic matter in the sediments is still unknown (CRRC 2010). During storms these sediments can be redistributed, but this is an infrequent event. In the Barrow area, separate zones of mostly pebbles and mostly sand alternate. If an oil spill occurs on such a beach, oil could penetrate up to 50 cm depth (pooling there) and storms can erode some surface-spilled oil (NOAA 2002).

Oil movement deeper into the sand renders degradation difficult (EPA 2014). Tilling could be used as an oil spill response at such beaches, as this will allow the escaped oil to re-emerge at the surface where microbes can degrade the oil easier since sufficient oxygen is available. Tilling accelerates physical, chemical and biological processes that would be absent or slower under natural conditions (Owens and Lee, 2003). A study was conducted on a soil plot where IF-30 intermediate fuel was used as a contaminant and the role of tilling and fertilizer addition was monitored. It was found that the tilled sediment with fertilizer showed the maximum oil degradation compared to untilled and unfertilized sediment (EPA 2014).

2.1.5 Role of seawater salinity

The Beaufort Sea, Chukchi Sea and North Aleutian Basin have water temperatures and salinity ranging from 5-10°C and 10-24 g/l in the summer (CRRC 2010).

The interaction of oil and minerals (present either in the sediments, soil or rocks) is an important factor in the clean-up of an oil spill. The formation of oil-mineral aggregates (OMA) helps in the degradation of oil. Recent studies have confirmed that saline seawater enhances the formation of OMA thus supporting the activity of biodegradation at higher salinity. However, under low saline conditions, some OMA formation still occurs (Owens and Lee, 2003).

Salinity is a major factor that affects microbial activity in the marine environment (Thavasi et al. 2007). High salinity can make the conventional bioremediation of oil difficult in crude oil contaminated water (Diaz et al. 2002). It was found that increased levels of salinity (33-282 g/l) decrease hydrocarbon degradation. Research showed that water with a salinity range of 0-60 g/l had maximum hydrocarbon degradation. A study was conducted by Diaz et al. (2002), where a bacterial consortium of MPD-M cells was immobilized on polypropylene fibers. These immobilized cells were used to degrade the oil in water with a salinity range of 0 to 180 g/l. The immobilized cells showed higher hydrocarbon degradation rates than non-immobilized cells (Diaz et al. 2002). This is a new approach to bioaugmentation (i.e. inoculation with microbes) in this case immobilizing microbes to increase the efficiency of degradation.

A positive correlation between salinity and the rate of PAH mineralization was also observed; 10 g/l and 30 g/l NaCl concentration facilitated degradation of PAHs in the soil (Minai-Tehrani et al. 2009), whereas 50 g/l NaCl inhibited the microbial activity. On the other hand, a strain of *Pseudomonas aeruginosa* showed the maximum biodegradation activity and growth at 35 g/l salinity compared to other high and low salinities (Thavasi et al. 2007).

Most of the above experimental studies used NaCl to prepare saline water. However one should note actual seawater contains other constituents in addition to NaCl. The above results from different experimental studies describe that salinity does have a significant effect on oil biodegradation, however results differ as to what salinity leads to the highest degradation rates.

2.1.6 Role of crude oil

Crude oil is a complex mixture of hydrocarbon compounds including alkanes, cycloalkanes and aromatic hydrocarbons (Zong et al. 2009, API 2011). A report submitted to the EPA in 2011 by the American Petroleum Institute (API) divides crude oil into three categories: light, medium and heavy crudes. Crude oil is classified by its density and API gravity is a common measure. The standard formula for API gravity calculation is API=141.5/Specific Gravity -131.5. Light crude has an API gravity $\geq 33°$; $\leq 28°$ API is considered heavy crude, and values in between are for medium crude oil.

The rate of crude oil biodegradation depends on the composition of hydrocarbon compounds present in the crude. Polycyclic aromatic hydrocarbons (PAHs) take longer than aliphatic compounds to degrade, also at cold temperatures (Zong et al. 2009). In decreasing order, the biodegradability of compounds at low temperature is n-alkanes, branched-chained alkanes, branch alkenes, n-alkyl aromatics, monoaromatics, cyclic alkanes and PAHs (Zong et al. 2009).

The crude oil concentration plays an important role in the act of degradation. A biodegradation study was conducted with substrate concentration (crude oil) varying from 0.1%- 4.5% in water. Maximum degradation activity was found at a substrate concentration of 2% in water samples (Thavasi et al. 2007). This experiment revealed the significance of oil concentration on its biodegradation.

Residual crude oil is no longer degradable and contains mainly PAH and asphaltenes. In a study conducted at Sorrizo beach which was affected by the Prestige oil spill, the rate of biodegradation of weathered fuel oil remaining after initial volatilization and some microbial degradation was monitored. Neither biostimulation nor bioaugmentation increased the residual fuel oil degradation (Fernández-Álvarez et al. 2006). However the introduction of biodiesel in addition to the weathered oil already present was able to increase the degradation of weathered oil (Fernández-Álvarez et al. 2006).

2.1.7 Role of evaporation

Weathering of oil in the water column includes surface evaporation, droplet formation, biodegradation and other environmental processes (Wrabel and Peckol, 2000, Brakstad and Bonaunet, 2006). At an oil spill site with low nutrient availability, natural attenuation proceeded mainly by evaporation and little microbial degradation (Wrabel and Peckol, 2000). However with the addition of nutrients like N and P in autoclaved seawater, 25-30% more n- alkanes were lost due to evaporation (Wrabel and Peckol, 2000). Low temperature usually results in reduced evaporation of volatiles, and in a delayed start of biodegradation (Heiser, 1999, Zong et al. 2009, Aislabie et al. 2006, Margesin, 2000). The volatilization of short-chain alkanes is higher at higher temperatures.

2.2 Research need

Since the environmental fate of crude oil depends on many factors including microbial activity, nutrient availability, temperature, sediment type, type of crude oil and salinity, the question arises to what extent volatilization and biodegradation contribute to crude oil removal in Arctic environments. While all the above environmental factors are relevant at the same time, within the scope of this thesis, the effect of temperature, salinity and crude oil concentration on degradation and volatilization rates was investigated in a controlled laboratory experiment. A microcosm experiment using sediments from Barrow, Alaska was designed to act as a proxy of an oil spill occurring on Alaska's Arctic coast.

Chapter 3 Material and Methods

3.1 Sampling site description

Sediment samples were collected in the summer of 2013 at the Barrow shoreline where the Chukchi Sea and Beaufort Sea meet. The air temperature during sample collection was 4°C. There are discrete areas of only sand or pebbles along with some mixed sand/gravel areas along the Barrow coastline. The sediment samples for this study were collected from the mixed areas. The coordinates of the sediment collection site is 71°21'39.80"N, 156°21'47.90"W. The collected material can be classified as ESI 5, which is explained in detail in section 2.1.4. Sediment for use in experiments was dug out up to a depth of 50 cm. These sediments along with seawater from this site were brought to the UAF campus.

The sediments were stored at 4°C to maintain the native conditions. The salinity of the sea water from the Chukchi Sea and Beaufort Sea was 30 g/l. There was some organic carbon content present in the sediments which was determined by gas chromatography/flame ionization (GC/FID), this is noted in the Figure 4.5 and Figure 4.12.

3.2 Experimental setup

The experiment was designed to approximate the environmental conditions of Barrow. Clear canning jars were filled with 1 kg of Barrow sediments, characterized using the ASTM C136-06 method. The sediment material was mixed to ensure that each jar received approximately the same material. Approximately 10 kg of sediment was autoclaved for sterilization to be used in the sterile control microcosms. In the experimental microcosms the sediment was not sterilized in order to evaluate biodegradation by the naturally present microorganisms. No inoculation was performed.

Figure 3.1 shows the experimental set up for this study. A metal stand prepared from a 12 inches long steel wire with a circular loop at the top was inserted in the sediments to suspend a beaker filled with 20 ml of 1 N NaOH solution in the headspace.

"Tea bags" of 10x8 cm, open on one side, were made from a mosquito net. Each bag was filled with 5 g of coconut shell activated carbon and suspended with a string in the jar's headspace to trap the volatile compounds released from the crude oil.

To each jar 10 ml of nutrient solution was added, which was prepared from fertilizer with an N/P/K ratio of 20/20/20, whereby the total nitrogen was 20% ammonia, 30% nitrate, and 50% urea nitrogen. Each microcosm received a total nitrogen concentration of 300 mg/kg sediment.

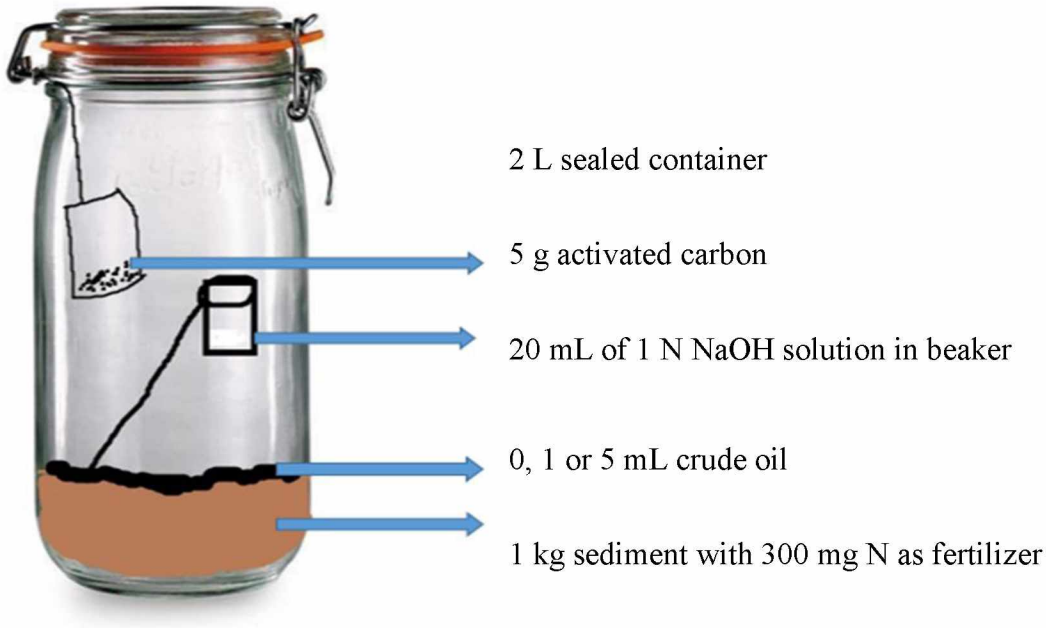

Figure 3.1 Illustration of microcosm containing sediments with crude oil

Quadruple microcosms were set up for seven different conditions, 3 control and 4 experimental, with varying crude oil concentrations and salinities as specified in Table 3-1. This means 28 jars were prepared for each temperature.

Table 3-1 Experimental parameters for microcosms at 20°C and 3°C

Type	Setup ID	Crude Oil (mL/kg)	Salinity (g/L)	Sterilized	No. replicate Jars
control	C0S1	0	30	--	4
	C0S1	0	30	yes	4
	C1S1	1	30	yes	4
experimental	C1S1	1	30	--	4
	C1S2	1	35	--	4
	C2S1	5	30	--	4
	C2S2	5	35	--	4

Sediments brought from Barrow had been naturally saturated with ocean water of a salinity of 30 g/l. This material was used for S1 (low salinity of 30 g/l) experiments. To increase the salinity for S2 (high salinity) experiments, sediment material were flushed with artificial seawater of 35 g/l salinity, prepared using Instant Ocean aquarium salt, before filling the jars.

Either 1 or 5 ml of crude oil was pipetted onto the sediment surface. The specific gravity of crude oil was determined to be 0.87 at 20°C and 3°C. Therefore the initial concentration of added crude oil was 870 mg/kg for 1 ml of crude oil added and 4350 mg/kg for 5 ml of crude oil. After addition of crude oil and fertilizer, the sediment was mixed vigorously with a spoon as a proxy for tilling of sediments in a real life scenario.

All jars were tightly sealed and placed inside the fume hood at 20°C for 6 weeks. In the 3°C study, all jars were kept in a refrigerator for 9 weeks.

3.3 Experimental sampling

The experiment was designed to establish a mass balance for the initially present crude oil, including volatilization of short chained hydrocarbons, crude oil remaining in the sediment, and crude oil mineralized (converted to carbon dioxide). The following parameters were determined to evaluate the results:

- CO_2 produced and captured in NaOH solution was quantified by titration with HCl.
- Volatiles released from the crude oil and captured by activated carbon were assessed via gas chromatography/mass spectrophotometry (GC/MS).
- Crude oil remaining in the sediments was determined by gas chromatography/flame ionization detector (GC/FID).
- The number of microbes present in the sediments was calculated by using the most probable number (MPN) method.

The methodology for each of these is described in detail in the following subsections. The sampling frequencies for 20°C and 3°C are shown in Table 3-2 and Table 3-3. As listed in Table 3-4, every two or three weeks, seven jars (one for each experimental condition) were sacrificed for sampling to determine TPH and the MPN of hydrocarbon degraders in sediments. Jars A, B, C and D are replicates i.e. for each environmental condition four replicates were available. Samples were stored at -80°C until analysis.

Table 3-2 Sampling frequency at 20°C

Parameter	Medium Sampled	Sample Size	Frequency	Duration
MPN	Sediment	1 g	Every two weeks	6 weeks
Crude Oil	Sediment	10 g	Every two weeks	6 weeks
Volatile	Activated Carbon	5 g	Once per week	6 weeks
CO_2	NaOH	20 ml	Once daily	Week 1-3
			Every two days	Week 4-6

Table 3-3 Sampling frequency at 3°C

Parameter	Medium Sampled	Sample Size	Frequency	Duration
MPN	Sediment	1 g	Every three weeks	9 weeks
Crude Oil	Sediment	10 g	Every three weeks	9 weeks
Volatile	Activated Carbon	5 g	Once per week	9 weeks
CO_2	NaOH	20 ml	Once daily	Weeks 1-3
			Every two days	Weeks 4-9

Table 3-4 Time of sacrificing replicate jars A, B, C and D for sampling

	A	B	C	D
All 20°C microcosms	Sacrificed after 6 weeks	Sacrificed after 6 weeks	Sacrificed after 4 weeks	Sacrificed after 2 weeks
All 3°C microcosms	Sacrificed after 9 weeks	Sacrificed after 9 weeks	Sacrificed after 6 weeks	Sacrificed after 3 weeks

3.4 Titration to determine CO_2 production

Carbon dioxide evolution, a proxy of microbial activity, was measured as described by Horel and Schiewer (2009). 20 ml of 1 N NaOH solution was placed in a beaker suspended in the head space of each microcosm. Every day or every second day the beaker was removed from the microcosm. Since microbial activity decreases with time, titrations were conducted every two days from the third week onwards. The NaOH solution was titrated against 1 N HCl solution in the presence of excess 0.3 N $BaCl_2$ solution. 1% phenolphthalein solution was used as a color indicator. A Metrohm titrino was used for conducting the titrations. The mass of carbon dioxide released in a day or two days, which describes the rate of microbial activity, was calculated from the equation below.

$$V_{titrant}(mL) \times C_{acid}\,(M) \times MW_{CO2} = m_{CO2}(mg)$$

3.5 Gas chromatography/flame ionization detection

The remaining crude oil in the sediments was determined using gas chromatography/flame ionization (GC/FID). Triplicate 10 g sediment samples were taken from each jar. Crude oil was extracted from sediment samples via 25 ml methylene chloride where 25 µl of D-5 nitrobenzene was used as an internal standard and 250 µl of D-8 naphthalene was as a surrogate. The standard concentration for D-5 nitrobenzene was 2500 mg/l and 2190 mg/l for D-8 naphthalene. The total petroleum hydrocarbons (TPH) in the sediments were computed using a modified AK 102 and AK 103 methodology.

An Agilent Technologies 6890N Network GC coupled with flame ionization detector with column parameters 30 m by 250 µm by 0.25 µm was used. The TPH method used a pulsed splitless injection with hydrogen as carrier gas (pressure 20 psi, flow 12.4 ml/min, average velocity 15.2 cm/sec). The oven temperature started at 40˚C increasing to 350˚C over duration of 34.50 minutes.

The total area of the chromatogram was taken into account when calculating the concentration of crude oil present in the sediments, using a standard calibration curve based on standards in the diesel range. The calibration standards were prepared over the range of 500-5000 mg/l for highly concentrated samples and 175-700 mg/l for samples with lower crude oil concentration. The standard concentrations were based on the theoretical initial crude oil concentration of 870 mg/kg for C1 and 4350 mg/kg for C2, considering that 1000 mg hydrocarbons per L of extract correspond to 2500 mg hydrocarbons per kg soil. The extraction efficiency of D-8 Naphthalene was calculated to be in the range of 80-95%. To obtain the actual TPH values, the measured sample TPH concentration was divided by the crude oil recovery factor (measured initial TPH concentration/theoretical initial crude oil concentration, which averaged 0.92). The sample concentration was calculated for days 0, 14, 28 and 42 of the 20°C study and days 0, 21, 42 and 63 of the 3°C study where the sampling frequency was 3 weeks (see Table 3-4).

3.6 Gas chromatography/mass spectroscopy

Volatile compounds released by crude oil were trapped in 5 g of activated carbon suspended in a mesh bag in the jar. In weekly intervals, the activated carbon was removed from the microcosms. To extract hydrocarbons from the activated carbon, 0.22 g of activated carbon was put in a GC vial and 1.5 ml of carbon disulfide was added with 25 µl of D-5 Nitrobenzene as an internal standard to verify the efficiency of the extraction. The concentration of the internal standard was 2500 mg/l.

To determine the gasoline range organics, the AK 102 method was modified. The GC-MS was an Agilent Technologies 6890N Network with a JW 123-1062 and a column of 60 m by 250 µm by 0.25 µm. The volatile organic compounds (VOC) method uses a splitless injection with helium as

carrier gas (pressure 9 psi, flow 1.6 ml/min, average velocity 3.2 cm/sec). The oven was set at an initial temperature of 150°C and increased to 350°C over 16.50 minutes.

A calibration curve was established using standards over a range from 250 to 5000 mg/l. The total area of the gasoline range was used and the concentration of the released volatiles was calculated based on the calibration curve. D-5 Nitrobenzene had an affinity for the activated carbon used in this study. Therefore, the recovery efficiency for the volatiles was compromised. In order to obtain the maximum recovery of the internal standard, the supernatant carbon disulfide solution containing the VOC was extracted in another vial. The VOC containing solution was then introduced with internal standard to monitor the recovery efficiency of the internal standard. The same experimental procedure and data analysis was followed for experiments at both 20°C and 3°C.

3.7 Most probable number

Crude oil is degraded by microbes present in the sediments. The number of crude oil degrading micro-organisms was calculated by using the most probable number (MPN) method. This technique follows a standard protocol where triplicate 1 gram sediment samples from each jar were taken in a falcon tube. Ten ml of 1% sodium pyrophosphate solution along with 3-4 grams of sterile glass beads were added to those falcon tubes. This mixture was put on the shaker table for 1 hour, after which the tube was allowed to stand for half an hour. Then, in each well of 96-well microtiter plates, 180 µl of Bushnell growth medium, 20 µl of microbial suspension (after 1.5 hours) and 5 µl of filtered crude oil (carbon source) was added. Three replicates (i.e. rows with increasing dilution level) for each sample were performed, with one control row containing no carbon source and one control row containing no microbial suspension, as shown in Figure 3.2. These 96-well plates were then incubated for 14 days at room temperature. Five µl of 2-(4-

iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium (INT) dye was added to each well on the 15th day and again the plates were incubated for 24 hours in the dark. Positive growth as indicated by a change in color (pink) was noted on the 16th day. This procedure was followed for the experiment conducted at 20°C.

The observed number of positives at each dilution was entered into the EPA MPN calculator software to determine the number of microbes. The EPA MPN calculator provides a specific concentration in MPN per milliliter of extract from the sediments based on the number of positive wells.

However there was some inconsistency in recording the color change on day 16, as the crude oil formed a dark layer on top of each well, hindering the correct color change identification. Therefore, the carbon source was changed for low temperature experiments.

For the 3°C experimental study, the same procedure was followed except that diesel was used as a carbon source instead of crude oil, and that the incubation temperature was 10°C. Crude oil or a refined petroleum product is used as a carbon source substrate to calculate the oil degrading bacteria (Wrenn and Venosa, 1996). Diesel, a refined product of crude oil, is clear in color, and was expected to produce a similar result as crude oil, but enabling better visual inspection. According to Wrenn and Venosa (1996), alternatively to crude oil as a carbon source two different carbon sources one for alkanes and other for PAHs can be used. The incubation temperature over 14 days was kept at 10°C as this was expected to result in a reasonable number of microbes for the reading on day 16.

Unfortunately, for both temperatures no reasonable MPN results were obtained, due to poor visual inspection at 20°C with crude oil as the carbon source, and low temperature dormancy of the

microbes. Therefore the MPN results will be not be discussed in the results section but are shown in Appendix A.

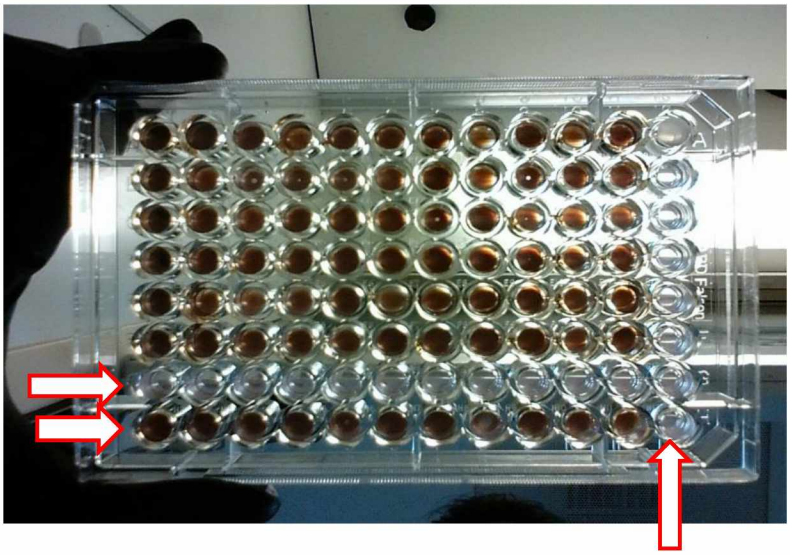

Figure 3.2 MPN plate with crude oil as C source at 20°C.

Chapter 4 Results and Discussion

As described in Table 3-1, there were 7 different setups including 3 controls and 4 experimental microcosms. The abbreviation Co specifies that no crude oil was added, C1 stands for a low crude oil concentration (1 ml/kg), C2 indicates high crude oil concentration (5 ml/kg), S1 refers to low salinity (30 g/l, as present in Beaufort and Chukchi sea) and S2 refers to a high salinity (35 g/l, as common worldwide). These abbreviations will be used throughout this thesis to refer to the results at 20°C and 3°C. These abbreviations are used in combination with each other since combinations of both parameters were studied at the same time.

4.1 Biodegradation of crude oil at 20°C

The rate of crude oil degradation for different salinities and crude oil concentrations was studied over a period of 6 weeks. Quantities of CO_2 evolution, remaining crude oil in sediments and volatiles released during that time are compared.

4.1.1 Carbon dioxide production

Measuring carbon dioxide production, i.e. respiration, is a primary method to determine the rate of biodegradation of hydrocarbons over time. Figure 4.1 shows the total CO_2 produced in each jar at different conditions over a period of 6 weeks. The following observations can be made from this figure.

1. Respiration for CoS1sterile was surprisingly higher than for CoS1. Also, the series for C1S1sterile and C1S1 overlapped. Apparently sterilization of sediments was not effective. As discussed in Appendix A, the MPN of hydrocarbon degraders in "sterile" microcosms were of similar magnitude as for unsterilized ones. Due to the lack of a biological hood and ineffective sterilization, the sediments in both C1S1sterile and C1S1 jars were exposed to similar

conditions (1 ml of crude oil, low salinity). Therefore, the CO_2 production was similar in both cases.

2. Respiration for C1S2 was higher than for C1S1, and similarly C2S2 produced more CO_2 compared to C2S1. This shows that higher salinity had a positive impact on CO_2 production or increased microbial activity.
3. The controls without any crude oil addition (Co) show a relatively high respiration, though a lower one than for C1 and C2. This points to another carbon source (beyond the added crude oil) being present in the sediment samples. This means only part of the CO_2 production can be attributed to crude oil degradation.

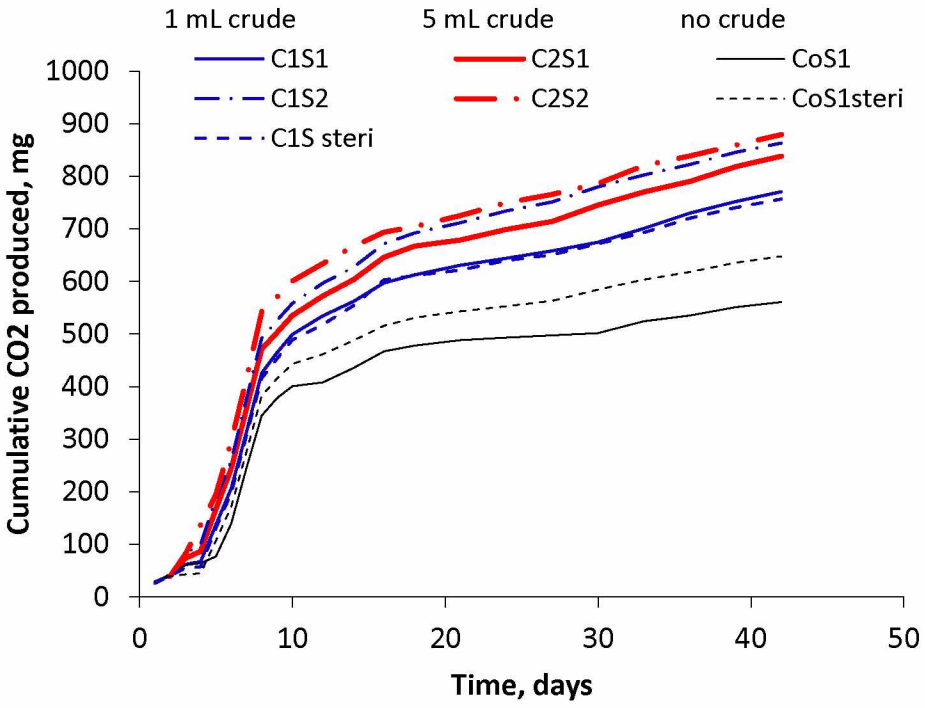

Figure 4.1 Cumulative CO_2 production at 20°C.

To better evaluate the impact of the added crude oil on respiration, the amount of CO_2 production due to crude oil mineralization was calculated by subtracting the CO_2 production for the corresponding microcosm without crude oil addition (Co) from the C1 or C2 microcosm with otherwise same conditions. Two figures were created based on the original respiration data from Figure 4.1. Figure 4.2 shows the CO_2 production due to crude oil degradation at low salinity (C1S1ster-CoS1ster, C1S1-CoS1 and C2S1-CoS1). In Figure 4.3 data for crude oil at high salinity are presented.

As depicted in Figure 4.2, C2S1 showed higher CO_2 production than C1S1 and C1S1sterile. Figure 4.3 shows the same trend at higher salinity; the production of carbon dioxide at high crude concentration was slightly higher than at low crude concentration. The higher the concentration of crude oil and salinity, the higher was the rate of CO_2 production and thus the rate of biodegradation.

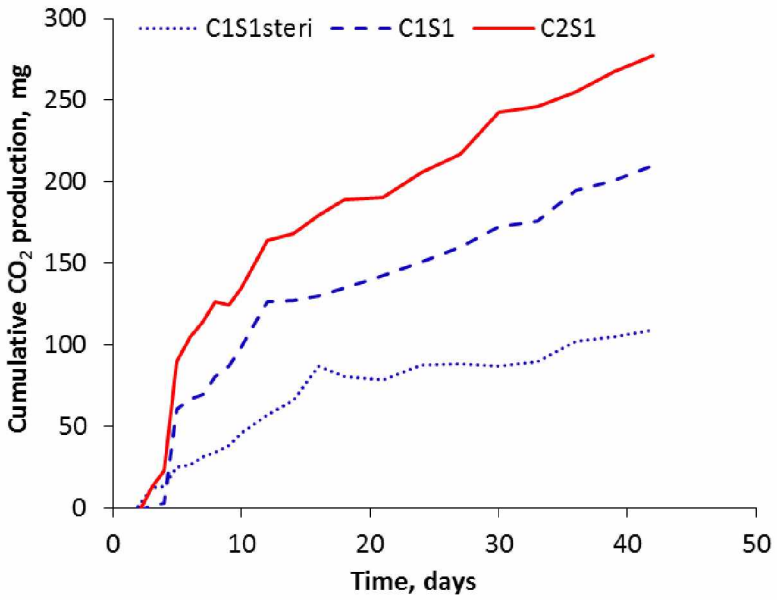

Figure 4.2 Cumulative CO_2 production due to crude oil for low salinity at 20°C.

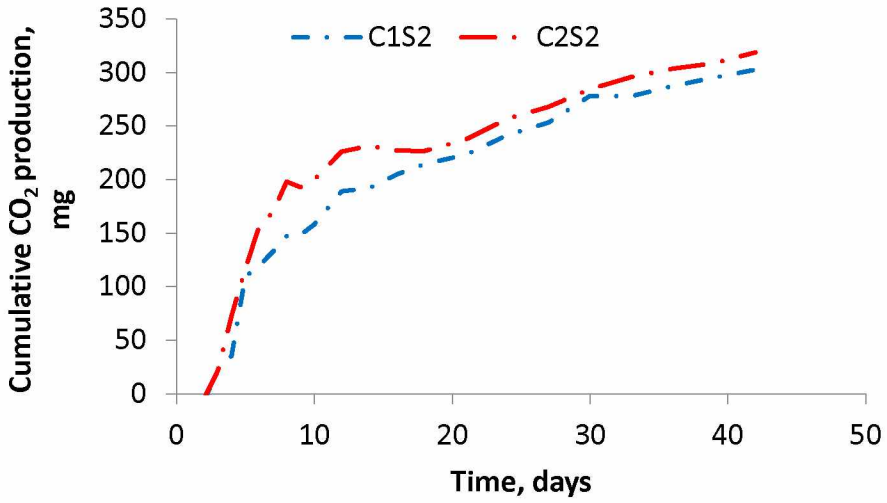

Figure 4.3 Cumulative CO_2 production due to crude oil for high salinity at 20°C.

4.1.2 Volatilization

Volatilization was measured in order to establish a mass balance for the added hydrocarbons. The Barrow shoreline experiences a lot of wind action, thus after an oil spill, wind action may carry away volatilized hydrocarbons, thus facilitating further volatilization, reducing hydrocarbon concentrations in the sediments at the spill site. The activated carbon included in the experimental setup acted as a sink, as a proxy for the wind removing volatilized hydrocarbon compounds. Figure 4.4 shows the amount of volatile compounds released per week from the crude oil during the 6 week incubation time, allowing the following observations:

1. The amount of volatiles released increased with the amount of crude oil present in the sediments.

2. Volatilization for microcosms without crude oil addition (CoS1 and CoS1sterile) was approximately zero for the first four weeks. Since there was no crude oil present in the sediments, no volatiles were released from the sediments. However volatilization increased

after 30 days to a low value of about 10 mg/week. This could either be due to some volatile compounds originating from crude oil stored in the fume hood during the experiment, or due to partial degradation of organic compounds creating more volatile products.

3. C1S1 sterile, C1S1 and C1S2 initially contained the same amount of crude oil and consequently showed very similar release of volatiles, which was only substantial over the first week and rapidly declined thereafter.

4. Similarly, C2S1 and C2S2 show similar volatilization due to the same amount of hydrocarbons being present. Volatilization was very high in the initial week and rapidly declined over the course of the experiment, with negligible volatilization after 2 weeks.

5. Salinity had no significant impact on volatilization.

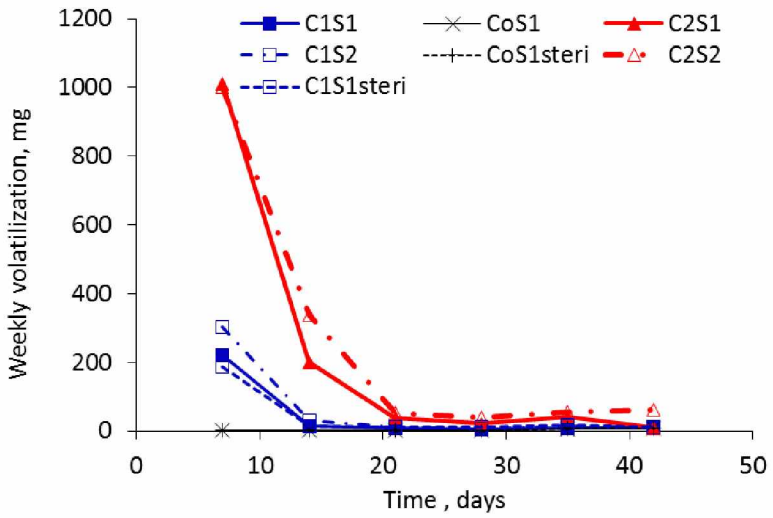

Figure 4.4 Amount of volatile compounds released per week from the crude oil at 20°C.

4.1.3 Hydrocarbons remaining in sediments

Total petroleum hydrocarbons present in the sediments declined over time at 20°C as illustrated in Figure 4.5, which shows the following trends.

1. The TPH measurements for the controls without crude oil addition (CoS1 and CoS1sterile) show that some organic compounds were initially present in the sediment. Some non-petroleum hydrocarbons can be extracted and detected using the TPH method used. This observation helps to explain the non-zero respiration shown for the controls in section 4.1.1.
2. The CoS1 and CoS1sterile series coincide, which can be explained by the fact that sterilization was not properly executed as discussed in section 4.1.1 and Appendix A.
3. C1S1sterile, C1S1 and C1S2 also show very similar results. Again, the fact that sterilization was not effective explains the similar behavior of the "sterile" setups. Salinity did not show a significant impact on TPH removal at low crude oil concentrations.
4. C2S2 and C2S1 initially showed a significant difference in the amount of crude oil measured though same amount of crude oil was added for both; the error bars range within 5%. This could be either a measurement error or an error when adding crude oil. TPH in C2S2 declined sharply and then showed similar values as for C2S1, which makes it more likely that a measurement error on day 1 occurred.
5. Hydrocarbon data in Figure 4.5 show a similar declining trend over time as volatile compounds in Figure 4.4.

Comparing Figure 4.1 through Figure 4.5 the same result was observed, that at high crude oil concentration and high salinity, maximum oil removal was observed.

The percentage of crude oil removal from the sediments over 6 weeks was calculated from the data in Figure 4.5 as % removal = $100 \times (TPH_{initial} - TPH_{day42})/TPH_{initial}$

The removal percentage shown in Figure 4.6, increased with increasing concentration and salinity following the same trends as discussed above. The average standard error for these samples was within 5%.

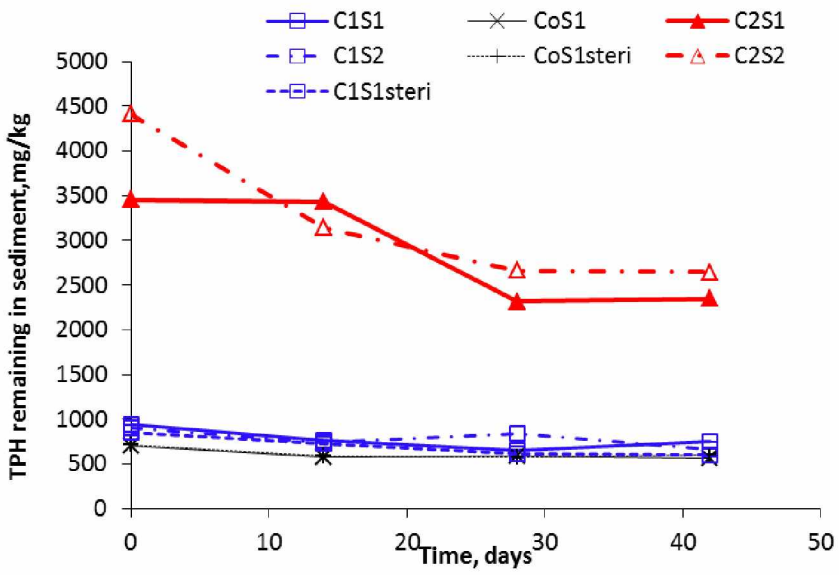

Figure 4.5 Total petroleum hydrocarbons remaining in sediments at 20°C.

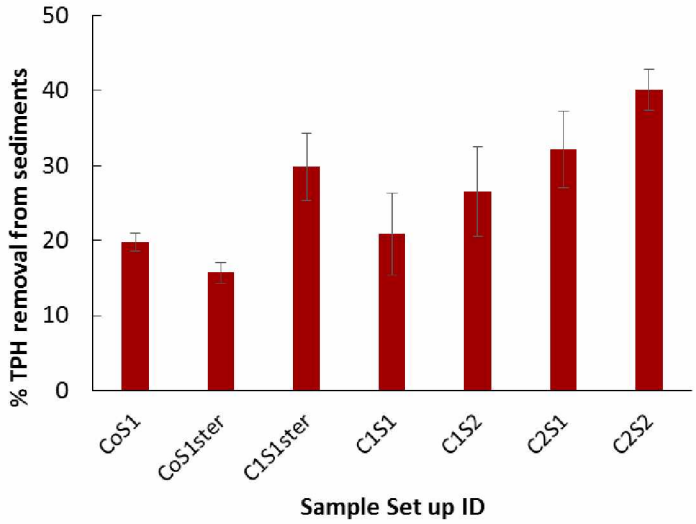

Figure 4.6 Percentage of TPH removal from sediments over 6 weeks at 20°C.

4.1.4 Mass balance of crude oil

A mass balance was made for the carbon initially present as crude oil, based on carbon recovery in the form of carbon dioxide, volatile compounds and total petroleum hydrocarbons present in the sediments.

The carbon from crude oil converted to CO_2 was calculated after subtracting CO_2 production for the Co control from the respiration for C1 and C2 samples. Since Co had some carbon content initially, C1 and C2 should have the same initial natural carbon content contributing towards CO_2 release. To relate the mass of hydrocarbons consumed to the amount of CO_2 produced in the experiment, the following generic stoichiometric equation for mineralization of hydrocarbons applies (Cunningham, 2004).

$$C_xH_y + \left[x + \left({}^{y}/_{4}\right)\right]O_2 \rightarrow xCO_2 + \left({}^{y}/_{2}\right)H_2O$$

For icosane, $C_{20}H_{42}$ as a representative TPH for the current study, the above equation can be written as:

$$C_{20}H_{42} + 30.5\ O_2 \rightarrow 20\ CO_2 + 21\ H_2O$$

This means the mineralization of 282 mg of hydrocarbons (1 mole icosane) leads to the production of 880 mg of carbon dioxide (20 moles), i.e. 0.32 mg crude were consumed per mg of CO_2 produced. In order to calculate what percentage of crude oil was converted to CO_2, the following equations were used:

$$m_{\,crude\ mineralized} = (m_{CO2} - m_{CO2\,Co}) \times 0.32\ \frac{g\ crude\ mineralized}{g\ CO_2\ from\ crude}$$

$$\%\ of\ crude\ converted\ to\ CO_2 = 100\ \times \frac{m_{\,crude\ mineralized}}{m_{\,crude\ added}}$$

For the amount of crude initially present ($m_{crude\ added}$), the theoretical value based on the volume of crude oil added was used, i.e. 870 mg for C1 and 4350 mg for C2.

In order to calculate the percentage of carbon initially present as TPH and eventually recovered as Volatile Organic Compounds (VOC), the following equation was used

$$\%\ of\ crude\ recovered\ as\ VOC\ = 100\ \times \frac{m_{volatiles}}{m_{crude\ added}}$$

The percentage of Total Petroleum Hydrocarbons (TPH) remaining in the sediment was calculated as

$$\%\ of\ crude\ remaing\ as\ TPH\ = 100\ \times \frac{TPH_{measured}}{TPH_{initial}}$$

The "total petroleum hydrocarbons" (TPH) measured according to the method used include diesel range organics, residual range organics, gasoline range organics, as well as naturally occurring oil and greases (EPA, 2014).

The mass balance for the initially present hydrocarbons consists of carbon dioxide released due to crude oil, TPHs and VOCs. The mass balance figure 4.7 shows the following trends:

1. Overall the fraction converted to CO_2 was the lowest compared to other mass balance fractions such as VOC and TPH. This implies that a very small percentage of crude oil was completely mineralized to CO_2. C2S1 and C2S2 showed the lowest percentage of CO_2 production with 2.1% and 2.4% respectively. The mineralization percentage for C1S2 was highest at 11.1%. This shows that at higher concentration of crude oil the microbes degrade a smaller fraction of the crude oil to CO_2. The mineralization percentage was slightly higher for S2, which indicates that increased salinity may enhance the CO_2 production.

2. Volatilization was generally quite high. The maximum percentage volatilized, 43 %, was obtained for C1S2. C1S1 and C2S1 produced 31% of volatile compounds. C2S2 released 36% volatiles. These percentages show that the crude oil concentration did not impact the volatilization percentage much but salinity could have an impact.

3. TPH remaining in the sediments were highest in C1S1 with 79.1% and C1S2 with 73.4%. C2S2 showed the minimum value with 60% of initially present hydrocarbons remaining.

4. Overall carbon recovery was around 100%, in one case as much as 128%. This over-recovery is likely due to the presence of naturally occurring carbon initially present in the sediments, which affected C1 microcosms more strongly than C2 microcosms, for which the natural carbon was a small amount compared to the large crude oil dosage.

These mass balance percentages provide a better understanding of what is actually happening and how much CO_2, VOC and TPH were recovered under different environmental conditions.

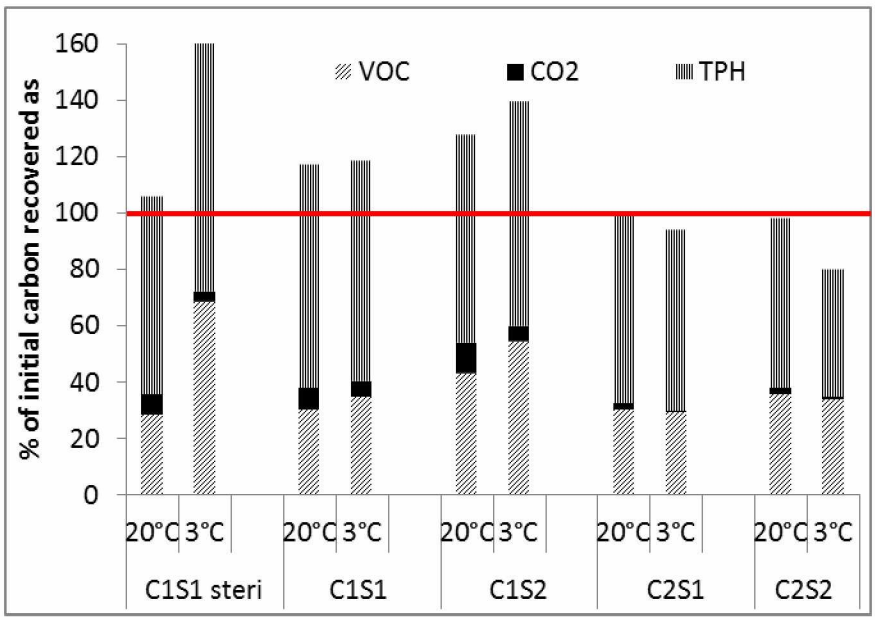

Figure 4.7 Mass balance of crude oil including fractions mineralized (CO_2), volatilized (VOC) and remaining in soil (TPH) at 20°C after 6 weeks.

4.2 Biodegradation of crude oil at 3°C

The same experiments were conducted at 3°C to study the effect of the same parameters at a lower temperature representative of typical summer temperatures in Barrow. Compared to the experiments at 20°C, the frequency of sampling was changed. Since the rate of biological activity declines with a decrease in temperature, the incubation time for this study was increased from 6 weeks at 20°C to 9 weeks at 3°C. Sampling frequencies were changed as described in Table 3-3.

4.2.1 Carbon dioxide production

In Figure 4.8, the cumulative respiration over time is shown, and the following observations can be made from this figure.

1. CoS1 and CoS1sterile series are overlapping, which was again due to ineffective sterilization. Both show substantial CO_2 production, in the same order of magnitude as other experiments with crude oil addition, even though both these jars are controls without addition of crude oil, and CO_2 production was expected to be low. Apparently some other carbon source was already present in the sediments. This matches observations made at 20°C. This can also be confirmed by data shown in Figure 4.8 where organic carbon was found in the sediments, even when no crude oil had been added.
2. All microcosms at the lower crude oil concentration, even the C1S1 sterile control showed higher CO_2 production than C2S2 and C2S1. This is unusual, typically CO_2 production increases with higher substrate (hydrocarbon) concentration. This could be because the microbes were not able to break down the complex compounds of crude into CO_2, inhibition or toxicity may have occurred at the higher crude oil concentration.
3. The low crude oil concentration series C1S1 and C1S2 are overlapping (no effect of salinity).

4. C2S2 showed higher CO_2 production compared to low salinity C2S1. Apparently higher salinity had a positive impact on CO_2 production for C2, as observed at 20°C.

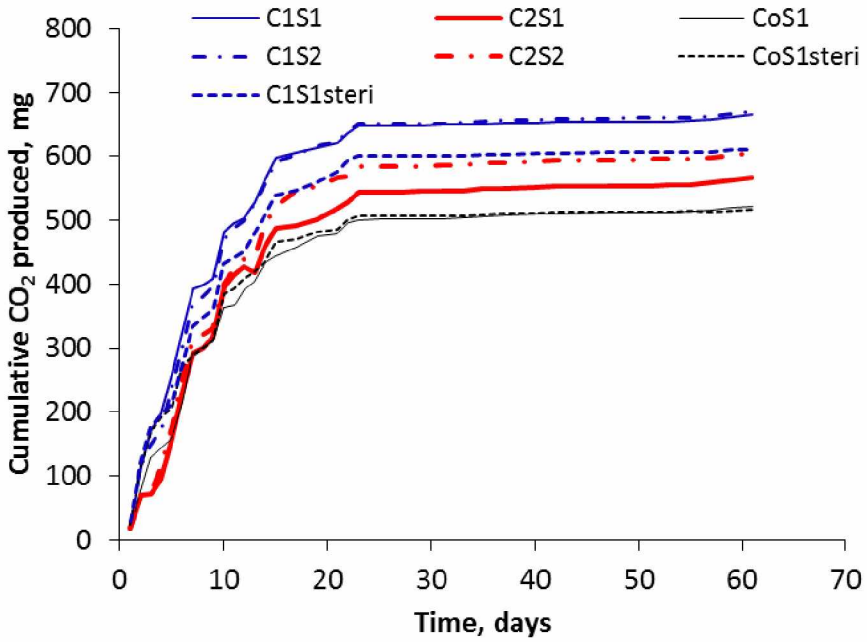

Figure 4.8 Cumulative CO_2 production at 3°C.

To compare the impact of the crude oil concentration and salinity, Figure 4.9 and Figure 4.10 show, at low and high salinity respectively, the CO_2 production due to crude oil mineralization for different concentrations after subtracting the CO_2 production for the control without crude oil from the data in Figure 4.8, similar as done for 20°C.

Figure 4.9 and Figure 4.10 show more clearly that high crude oil concentrations (C2) lead to lower CO_2 production than for low crude oil concentration (C1). At 3°C, the higher crude oil concentration apparently inhibited the process of CO_2 production. Some initial negative values (not shown) were calculated since Co had initially higher respiration than C1 or C2 in some cases. A possible explanation could be that crude oil hindered oxygen supply.

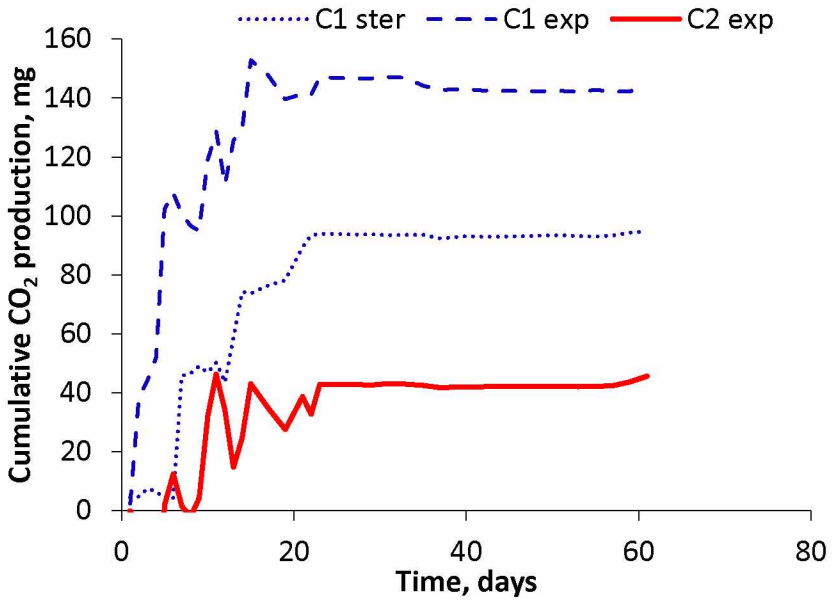

Figure 4.9 Cumulative CO_2 production due to crude oil for low salinity at 3°C.

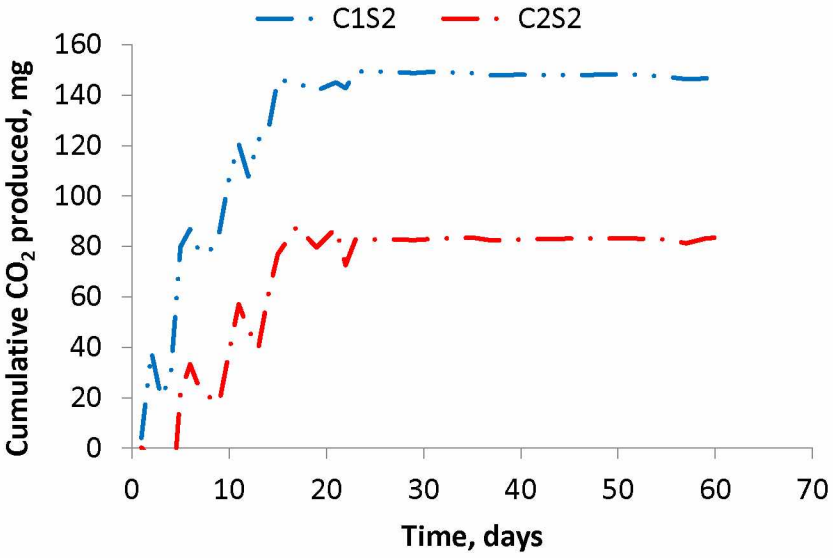

Figure 4.10 Cumulative CO_2 production due to crude oil for high salinity at 3°C.

4.2.2 Volatilization

The following observations can be made based on Figure 4.11 which presents the amount of volatiles released from crude oil over a period of 9 weeks at 3°C.

1. The amount of volatiles released increased with the amount of crude oil present.
2. CoS1 and CoS1sterile volatilization values were insignificant for the first four weeks. Since there was no crude oil present in the sediments, no volatiles were released from the sediments.
3. In all setups with 1 mL crude oil, (C1S1 sterile, C1S1 and C1S2) volatile compounds released declined over time, with similar volatilization values for all three series.
4. Similarly, the two setups with the higher crude oil concentration, C2S1 and C2S2, showed initially high volatilization with a decreasing trend, though some fluctuations were observed esp. around day 21.
5. Salinity had no significant impact on volatilization.

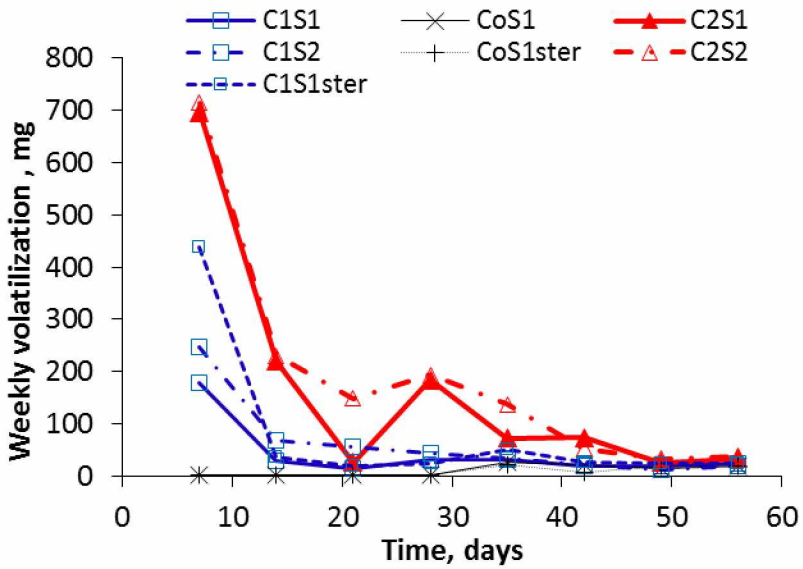

Figure 4.11 Amount of volatile compounds released per week from the crude oil at 3°C.

4.2.3 Hydrocarbons remaining in sediments

Total petroleum hydrocarbons present in the sediments at 3°C declined over time as illustrated in the Figure 4.12. For data obtained at different crude oil concentrations, the following observations were made.

1. The amount of TPH remaining in the sediment was higher for the higher crude oil dosage (C2).
2. TPH in the CoS1 control and CoS1 sterile were very low as expected. The small amount of TPH measured may be due to measurement error or other carbon compounds interfering with the measurement, as similarly observed for 20°C.
3. All setups at the lower crude oil concentration, i.e. C1S1 sterile control, C1S1 and C1S2 showed very similar and quite low TPH values, with little change over time. Salinity did not show a significant impact at low crude oil concentrations. Sterilization also showed no effect.
4. C2S2 and C2S1, which had same higher amount of crude oil, initially showed a difference, however TPH values for C2S2 declined sharply, eventually approaching C2S1 values which tapered off slowly over time.

The percentage of TPH reduction in sediments, calculated as described in section 4.1.3, is shown in Figure 4.13, allowing the following observations.

1. The TPH removal percentage increased with increasing TPH levels.
2. TPH in CoS1sterile and CoS1 sediments showed only little reduction of the already very low carbon levels.
3. The C1S1 "sterile" control showed comparable results to C1S1 and C1S2.

4. The maximum TPH reduction over 9 weeks occurred in C2S2. Higher salinity had a positive impact on crude oil degradation the higher oil concentrations; however there was very little impact of salinity at the lower crude oil dosage.
5. When Figure 4.8 and Figure 4.12 are compared, different conclusions can be drawn for C2S2 at 3°C. In Figure 4.8 C2S2 showed lower CO_2 production compared to C1S1, on the other hand in Figure 4.13 C2S2 revealed the maximum TPH reduction percentage. This means that crude oil removal was not directly linked to mineralization, requiring other explanations. To better understand the fate of the crude oil, a mass balance has to be established. The standard error of the samples was within 6% for the Figure 4.12.

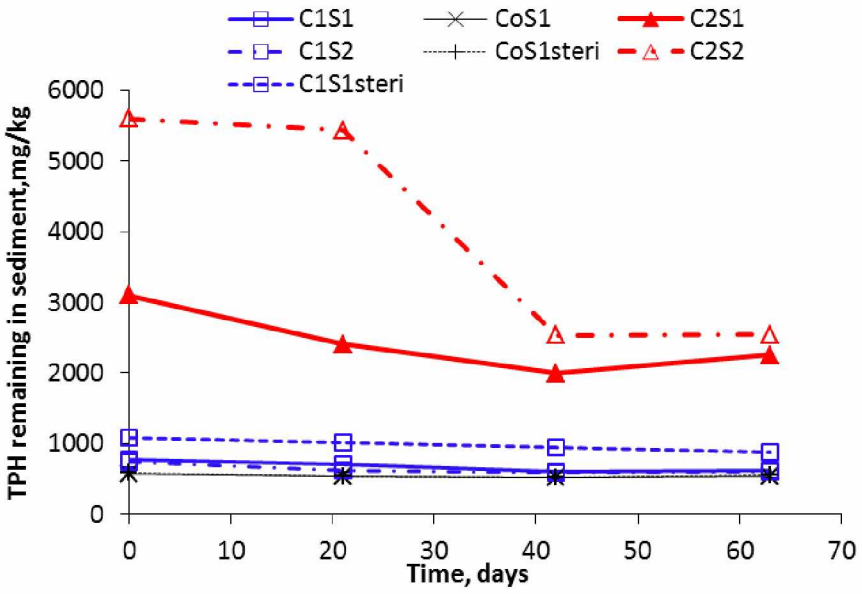

Figure 4.12 Total petroleum hydrocarbons remaining in sediments at 3°C.

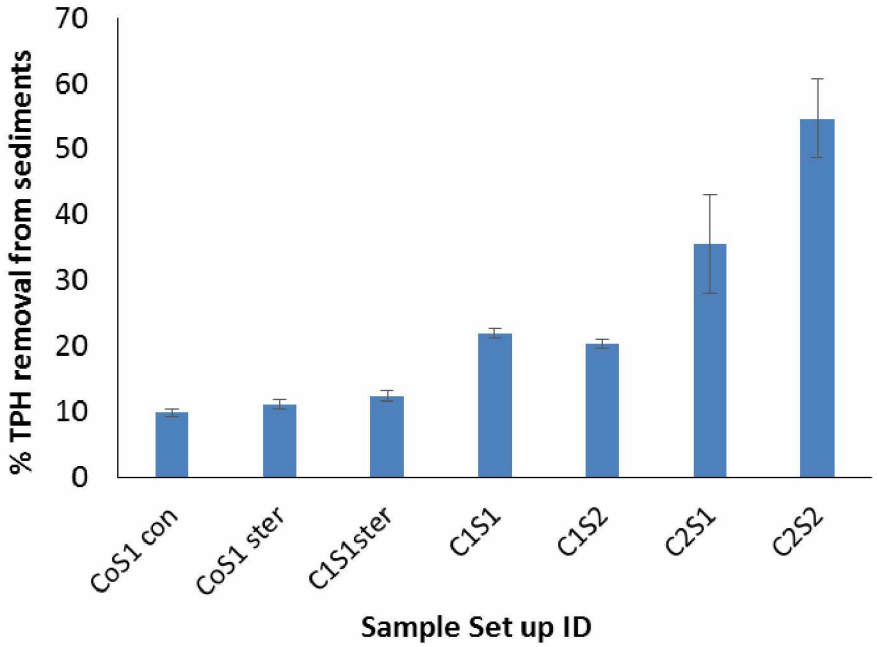

Figure 4.13 Percentage of TPH removal from sediments over 6 weeks at 3°C.

4.2.4 Mass balance of crude oil

Figure 4.14 shows how different conditions affected the fate of crude oil carbon after 9 weeks at 3°C. Mass balance percentages were calculated according to the same method as used at 20°C and described in section 4.1.4.

1. Overall mineralization was the lowest fraction, compared VOC and TPH. This means that a very small percentage of crude oil was completely mineralized to CO_2. C2S1 and C2S2 showed the lowest percentage of CO_2 production of 0.3% and 0.6% respectively, while C1S1 and C1S2 had 5.2% and 5.4% mineralization respectively. This shows that at higher crude oil concentrations a lower fraction was completely degraded by microbes.

2. At high concentrations of crude oil, a lower percentage was volatilized. The maximum volatilization percentage, 69%, was for C1S1sterile. Both S2 samples produced a higher percentage of volatile compounds.
3. The fraction of TPH remaining in the sediments was highest for C1S1 sterile control with 87.6%. C2S2 showed the minimum TPH remaining in the sediments. Thus, higher crude oil concentration and high salinity had a positive role in crude oil removal.
4. The overall mass balance recovery percentage was higher than 100% because of the initial organic carbon present in the sediments.

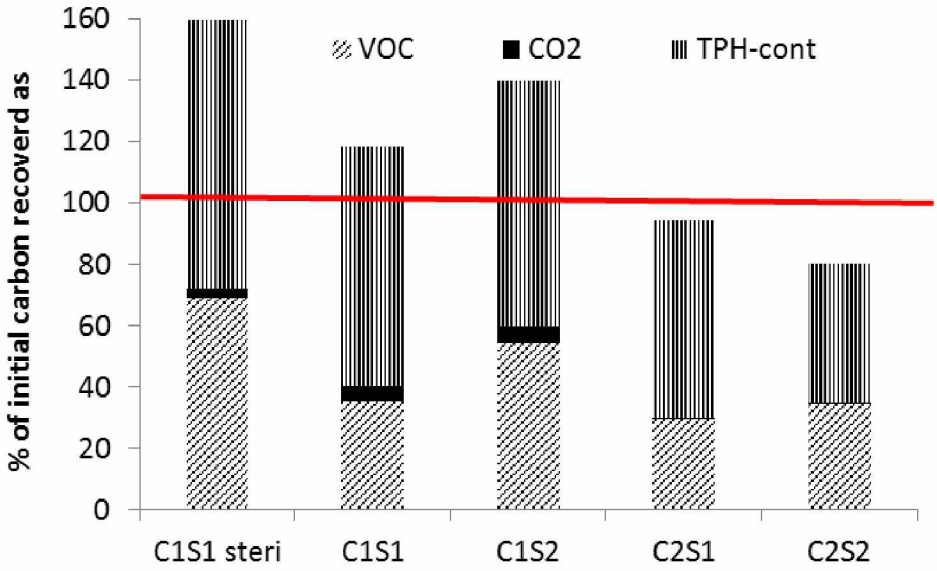

Figure 4.14 Mass balance of crude oil including fractions mineralized (CO_2), volatilized (VOC), and remaining in soil (TPH) at 3°C after 6 weeks.

4.3 Comparison between 20°C and 3°C

The experiments were designed to compare two different temperatures, 20°C and 3°C. According to the literature, the rate of hydrocarbon degradation typically increases with temperature, salinity and crude oil concentration. The impact of salinity on the rate of crude oil degradation at low temperatures has not yet been studied prior to the current project. Therefore, it is important to compare the results at 20°C and 3°C.

Since 20°C experiments were conducted for 6 weeks and 3°C studies were carried out for 9 weeks, the results for both temperatures will be compared over 6 weeks.

4.3.1 Carbon dioxide production

The following observations can be made from Figure 4.15 which shows the cumulative CO_2 production over time.

1. For each set of conditions, the CO_2 production at 20°C was higher than at 3°C. These results are consistent with the Arrhenius principle, according to which reactions slow down at low temperatures. Therefore more time will be required for the microbes to mineralize crude oil at lower temperatures.
2. For both temperatures and both crude oil concentrations (C1S1 vs. C1S2, and C2S1 vs. C2S2), respiration for S2 was higher than for S1. There may be a positive impact of higher salinity on the degradation of crude oil to CO_2.
3. At 20°C, respiration was a little higher for C2 than for C1, as is commonly observed. At 3°C, however, C2 showed a lower value than C1. This indicates that higher crude concentration inhibits the conversion of crude to CO_2 at 3°C.

4. For both temperatures C1S1sterile and C1S1 show similar values. Similarly, CoS1 and CoS1sterile are similar. This proves that sterilization was not effective.

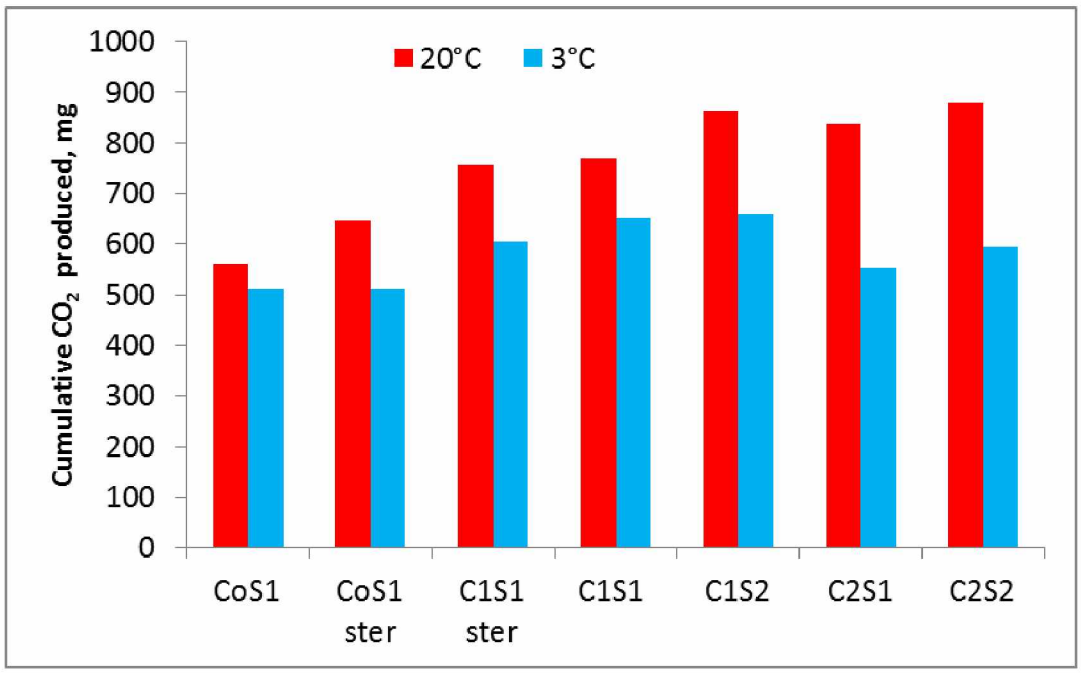

Figure 4.15 Cumulative CO_2 produced in 6 weeks at 20°C and 3°C

4.3.2 Volatilization

Figure 4.16 compares volatilization of organic compounds at both temperatures. The percentage volatilized was calculated as described in section 4.1.4.

1. According to literature, volatilization is slower at low temperatures. The data show however that the percentages of VOC at both temperatures were in most cases very similar.
2. C1S1sterile at 3°C even showed the maximum volatilization percentage of all setups, higher than any volatilization observed at 20°C.

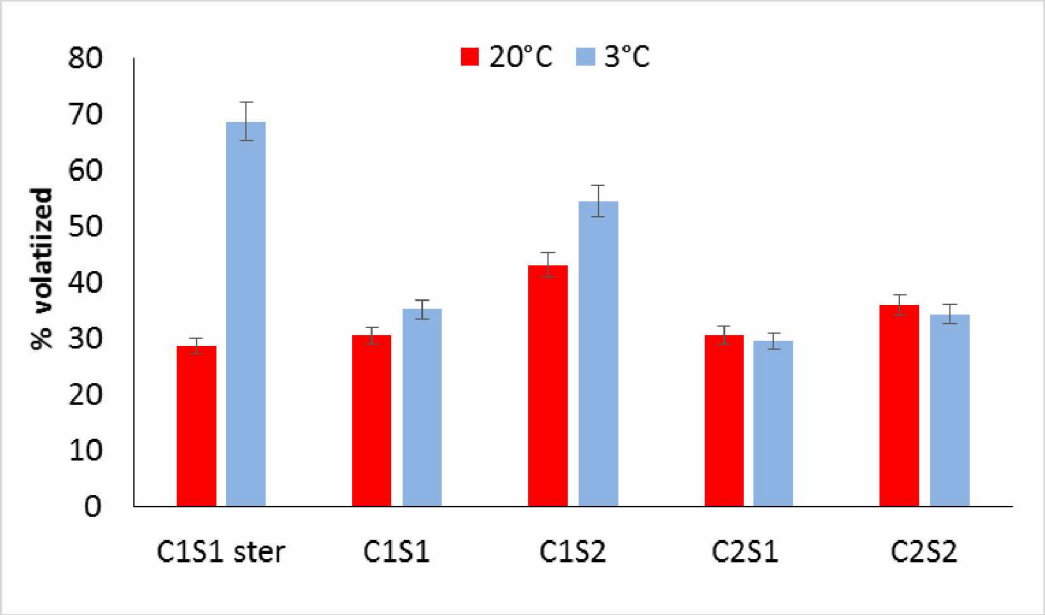

Figure 4.16 Percentage of volatile compounds released during 6 weeks at 20°C and 3°C

4.3.3 Hydrocarbons remaining in sediments

Figure 4.17 compares the percentage of total petroleum hydrocarbons remaining in the sediments at 20°C and 3°C after 6 weeks. The following observations can be made from this figure.

1. Similarly high remaining percentages of TPH were found at both temperatures
2. Higher salinity samples generally had lower remaining TPHs compared to low salinity, confirming the original hypothesis.
3. At both temperatures C2S2 had the lowest amount of TPH remaining at 3°C less than 50% remained after 6 weeks.

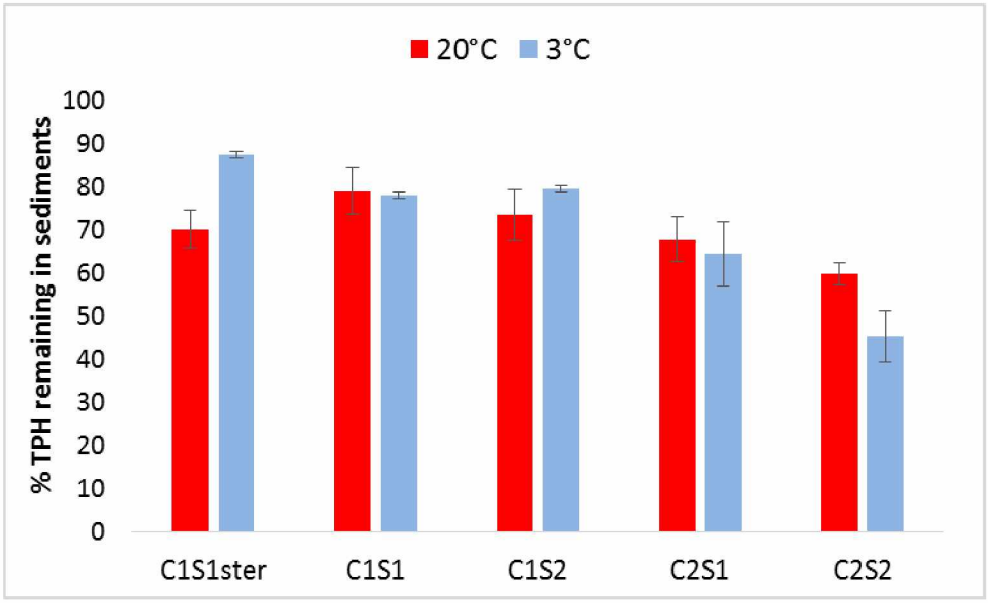

Figure 4.17 Percentage of TPH remaining in sediments after 6 weeks at 20°C and 3°C

4.3.4 Mass balance of crude oil

Mass balances for both temperatures are compared in Figure 4.18, allowing the following observations

1. The mineralization percentage "CO_2" at 20°C was consistently higher than at 3°C. This conforms to the Arrhenius relationship according to which rates increase with temperature.
2. According to the literature VOC release should slow down at low temperature. However most microcosms, especially C1S1 sterile, showed higher volatilization at 3°C than at 20°C. VOC releases in other microcosms were similar for both temperatures.
3. The remaining TPH percentage did not show a clear trend with respect to temperature; in some microcosms the remaining TPH were higher at 3°C, in other at 20°C.
4. Over-recovery exceeding 100 % occurred for C1 at both temperatures but not for C2. The naturally present carbon apparently contributed to the mass balance for low crude oil dosages but not for high crude oil dosages where it only constituted a relatively small percentage.

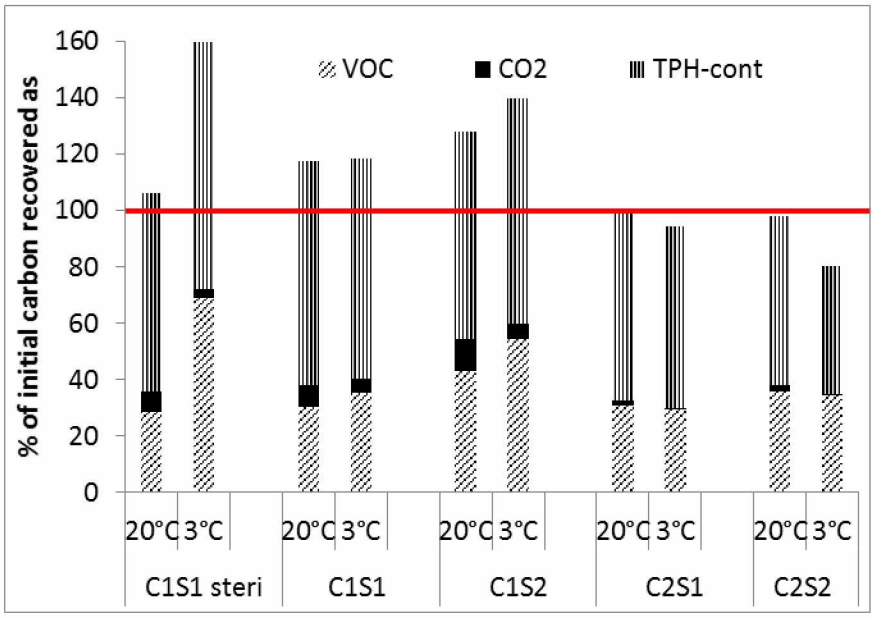

Figure 4.18 Mass balances of crude oil at 20°C and 3°C after 6 weeks.

Chapter 5 Conclusions

Conclusions of this research are based on the experimental results which either verify or contradict the hypotheses.

Controls without crude oil addition

There was some carbon naturally present in the Barrow sediments. Therefore, CoS1 and CoS1sterile, both of which had no crude oil added, showed substantial CO_2 release. CoS1 released 560 mg CO_2 at 20°C and 522 mg CO_2 at 3°C respectively.

Effect of microbes

Crude oil degradation by naturally present microbes occurred, as evident from CO_2 production, confirming hypothesis 1.

The CoS1 sterile control released a similar quantity of carbon dioxide (648 mg CO_2 at 20°C and 516 mg CO_2 at 3°C) as the unsterilized control. Evidently, sterilization was not effective. MPN measurements tentatively confirmed similar numbers of hydrocarbon degraders for sterilized and non-sterilized microcosms. It is advisable to use a biological hood to prevent cross contamination.

Effect of crude oil concentration

Samples with higher crude oil concentration did not produce a significantly higher amount of CO_2 at either temperature. At 3°C, mineralization was even somewhat lower for C2 than for C1, showing that complete mineralization is difficult at higher contamination levels. Hypothesis 2a, which stated that a higher crude oil concentration would increase the rate of mineralization was therefore proven wrong. In the mass balance relative mineralization percentages were lower for higher crude oil concentrations confirming the initial hypothesis 2b.

VOC release was higher for C2 than for C1, i.e. more volatilization took place at higher crude oil dosages, confirming hypothesis 3, and however the volatilization percentage was lower for C2.

At higher crude oil dosages, more TPH remained in the sediments, both in absolute and relative terms, confirming hypothesis 4.

Effect of temperature

Temperature played an important role in mineralization of crude oil. At 20°C higher CO_2 production was observed in than at 3°C, confirming hypothesis 5.

Surprisingly, similar volatilization was noted for both temperatures, contradicting hypothesis 6; literature describes that volatilization is lower at low temperatures (Margesin, 2000).

The amount of TPH remaining in the sediment was similar at both temperatures, especially for disproving hypothesis 7 that higher temperatures lead to better crude oil removal.

Effect of salinity

According to hypothesis 8, salinity has a positive impact on hydrocarbon degradation rates, which was confirmed by the results at 20°C and 3°C. At both temperatures, S2 samples displayed higher respiration and volatilization as well as lower TPH. Literature supports that at high temperature increased salinity leads to higher oil degradation rates. Based on the present research it can be concluded that the same effect can be seen at lower temperature as well.

Mass balance

According to the mass balance, only a small fraction (< 10%) was mineralized, most TPH removal was due to volatilization (~ 40%) rather than biodegradation. The mass balance percentages were higher than 100% due to the presence of organic matter in the sediments.

Overall it can be concluded that environmental factors like temperature, crude oil concentration and salinity all impact the rate of crude oil degradation in laboratory experiments and are expected to do so in the real world oil spill accidents.

Recommendations for future research

To better understand the influence of salinity, crude oil concentration and temperature, experiments with longer incubation period could be performed. A longer timeframe will help to determine what quantities of TPH would remain in the sediments in the long run.

The contribution of the naturally present carbon source should be better characterized.

Nutrient addition in regular time intervals might help maintain the microbial population in the exponential growth phase. This could increase the rate of degradation.

A different technique should be used for monitoring the number of microbes present in the sediments to provide an accurate number. Diesel as a carbon source in combination with incubation at 20°C, and a larger number of dilution steps could lead to better results. Also, initial MPN should be determined.

It would be useful to perform experiments with saturated versus unsaturated sediments and investigate the effect of saturation level on oxygen supply (aerobic vs anoxic) and resulting degradation rates.

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Appendix A MPN results

MPN results were obtained using the EPA MPN calculator after visual identification of positive wells. As shown in Figure A.1, the data do not show a clear trend over time, and do not follow the growth kinetics discussed in section 2.1.1.

The "sterile" microcosms contained similar quantities of hydrocarbon degraders as the other microcosms. This means sterilization by autoclaving had no lasting effect. Either the autoclaving was not effective in the first place, and/or microbes present in the ambient air colonized the microcosms. The latter is quite likely since no biological hood was available for these experiments. During daily CO_2 measurements, microcosms had to be opened, which could have allowed microbes to enter the sterilized microcosms.

Table A-1 MPN values of hydrocarbon degrader numbers per ml of soil extract at 20°C

Days	CoS1 con	CoS1 ster	C1S1 ster	C1S1 exp	C1S2 exp	C2S1 exp	C2S2 exp
	MPN/ml	MPN/ml	MPN/ml	MPN/ml	MPN/ml	MPN/ml	MPN/ml
14	0	370	11989	1053	466	9328	0
28	0	466	119893	734	119893	7344	360
42	0	105	7344	10532	1894	1914	310

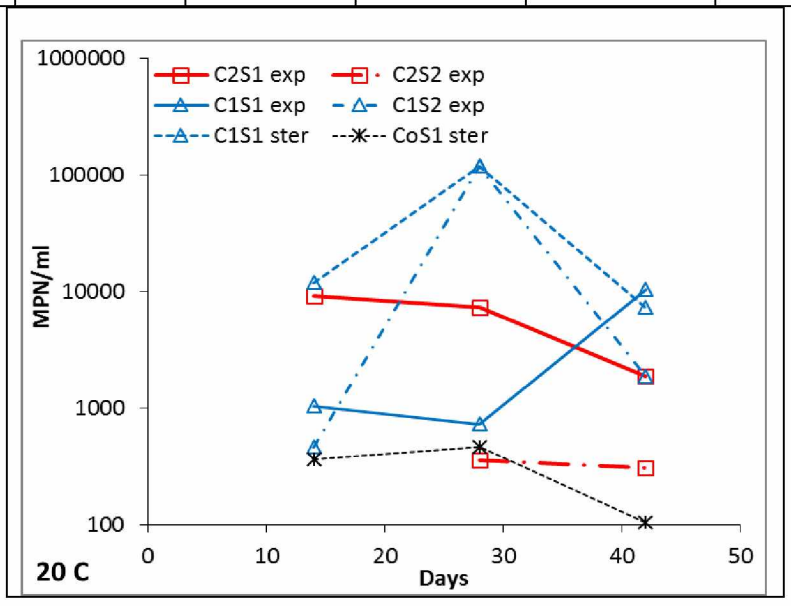

Figure 6.1 MPN of crude oil degraders from 20°C experiments, for different experimental durations, concentrations and salinities.

A further potential error source was the difficulty of identifying the color change indicating positive samples. Figure A.2 shows an MPN plate using crude oil as a carbon source at 20°C. Due to the presence of a dark surface layer of crude oil, it was difficult to visually identify the positive wells.

Therefore, diesel was used a carbon source for 3°C study. An example of a well plate using samples from 3°C experiments incubated at 10°C with diesel as a carbon source is shown in Figure A.3. A temperature of 10°C was used to incubate well plates from the 3°C experimental study due to the concern that incubation at 3°C may not result in color change within the incubation period. However, even at 10°C, only one well developed a pink color (i.e. positive for diesel degraders). Therefore, no MPN can be reported for the 3°C experiments.

The absence of color change in wells could be due to the lower temperature where the microbes would not yet degrade the diesel in the Bushnell medium since lag phases are typically longer at low temperature (Horel and Schiewer. 2011). It is likely that microbes were inactive during the incubation period, because after their incubation at low temperature, these plates were moved to a hazardous waste cabinet at room temperature, where all wells changed to a pink color within a few days. This shows that microbes must have been present and those microbes became active after being discarded, consumed the diesel, and caused a color change. Therefore no data are available for 3°C. For future studies it is recommended to change the protocol, e.g. by using a longer incubation period or a higher incubation temperature to ensure that microbes become active during incubation.

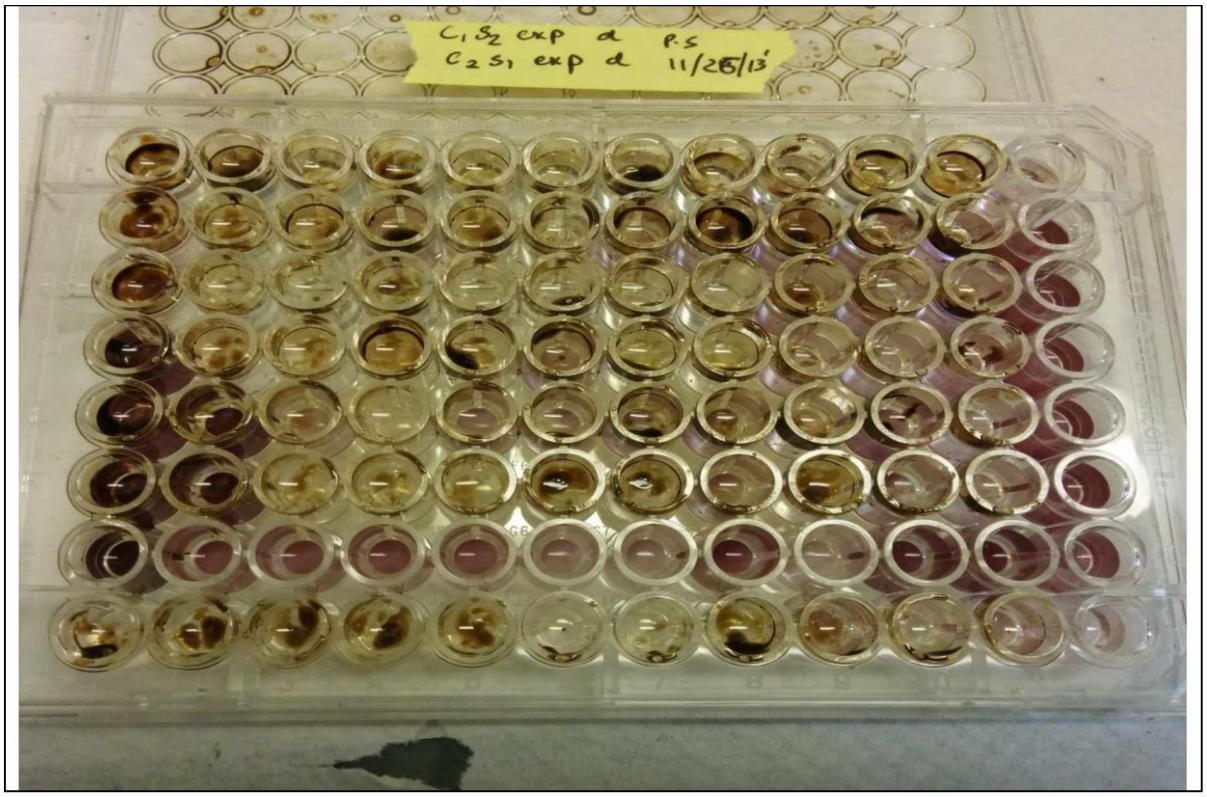

Figure 6.2 MPN plate for the C1S2 and C2S1 samples at 20°C with crude oil as C source

Figure 6.3 MPN plate for C2S1 and C2S2 samples at 3°C with diesel as C source